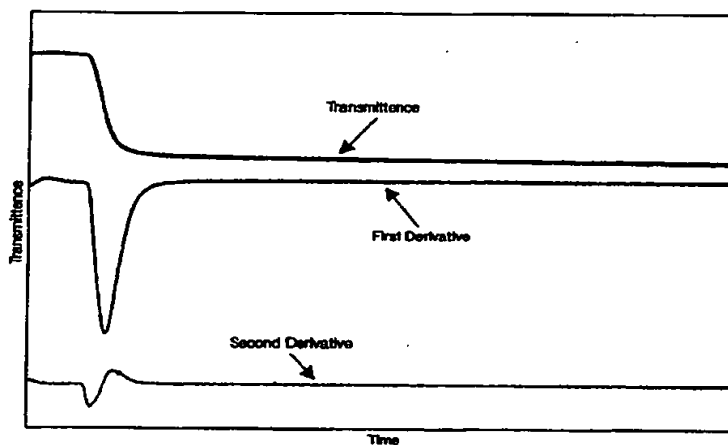


PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : G06F 19/00	A1	(11) International Publication Number: WO 96/41291 (43) International Publication Date: 19 December 1996 (19.12.96)
<p>(21) International Application Number: PCT/US96/08905</p> <p>(22) International Filing Date: 5 June 1996 (05.06.96)</p> <p>(30) Priority Data: 08/477,839 7 June 1995 (07.06.95) US</p> <p>(60) Parent Application or Grant (63) Related by Continuation US 08/477,839 (CIP) Filed on 7 June 1995 (07.06.95)</p> <p>(71) Applicant (for all designated States except US): AKZO NOBEL N.V. [NL/NL]; Velperweg 76, P.O. Box 186, NL-6800 LS Arnhem (NL).</p> <p>(72) Inventors; and (75) Inventors/Applicants (for US only): GIVENS, Thomas, B. [US/US]; 3522 Red Mountain Road, Rougemount, NC 27572 (US). BRAUN, Paul [US/US]; 612 Kingsbury Drive, Durham, NC 27712 (US). FISCHER, Timothy, J. [US/US]; 6405 Pernod Way, Raleigh, NC 27613 (US).</p> <p>(74) Agents: BLACKSTONE, William et al.; Akzo Nobel Patent Dept., 1300 Piccard Drive #206, Rockville, MD 20850 (US).</p>		<p>(81) Designated States: AU, CA, FI, JP, KR, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).</p> <p>Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i></p>

(54) Title: A METHOD AND APPARATUS FOR PREDICTING THE PRESENCE OF CONGENITAL AND ACQUIRED IMBALANCES AND THERAPEUTIC CONDITIONS



Normal APTT Optical Profile with First and Second Derivative

(57) Abstract

A method and apparatus are disclosed for predicting the presence of at least one congenital or acquired imbalance or therapeutic condition associated with thrombosis/hemostasis from at least one time-dependent measurement profile, (Figs. 3-6). At least one time-dependent measurement on an unknown sample is performed and a respective property of said sample is measured over time so as to derive a time-dependent measurement profile, (Figs. 3-6). A set of a plurality of predictor variables are defined which sufficiently define the data of the time-dependent measurement profile, (Fig. 13). A model is then derived that represents the relationship between the congenital or acquired imbalance or therapeutic condition, and the set of predictor variables. Subsequently, the model is utilized to predict the existence of the congenital or acquired imbalance or therapeutic condition in the unknown sample.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgyzstan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	KZ	Kazakhstan	SG	Singapore
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovakia
CM	Cameroon	LR	Liberia	SN	Senegal
CN	China	LT	Lithuania	SZ	Swaziland
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	LV	Latvia	TG	Togo
DE	Germany	MC	Monaco	TJ	Tajikistan
DK	Denmark	MD	Republic of Moldova	TT	Trinidad and Tobago
EE	Estonia	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	UG	Uganda
FI	Finland	MN	Mongolia	US	United States of America
FR	France	MR	Mauritania	UZ	Uzbekistan
GA	Gabon			VN	Viet Nam

-1-

**A Method and Apparatus for Predicting
The Presence of Congenital and Acquired
Imbalances and Therapeutic Conditions**

5

BACKGROUND OF THE INVENTION

10

This application is a continuation-in-part of U.S. patent application 08/389,986 to Fischer et al. filed February 14, 1995, the subject matter of which is incorporated herein by reference. This application is also related to the following publications, the subject matter of each also being incorporated herein by reference:

1. B. Pohl, C. Beringer, M. Bomhard, F. Keller, The quick machine - a mathematical model for the extrinsic activation of coagulation, *Haemostasis*, 24, 325-337 (1994).

2. J. Brandt, D. Triplett, W. Rock, E. Bovill, C. Arkin, Effect of lupus anticoagulants on the activated partial thromboplastin time, *Arch Pathol Lab Med*, 115, 109-14 (1991).

3. I. Talstad, Which coagulation factors interfere with the one-stage prothrombin time?, *Haemostasis*, 23, 19-25 (1993).

4. P. Baumann, T. Jurgensen, C. Heuck, Computerized analysis of the in vitro activation of the plasmatic clotting system, *Haemostasis*, 19, 309-321 (1989).

5. C. Heuck, P. Baumann, Kinetic analysis of the clotting system in the presence of heparin and depolymerized heparin, *Haemostasis*, 21, 10-18 (1991).

6. M. Astion and P. Wilding, The application of backpropagation neural networks to problems in pathology and laboratory medicine, *Arch Pathol Lab Med*, 116, 995-1001 (1992).

SUBSTITUTE SHEET (RULE 26)

-2-

7. M. Astion, M. Wener, R. Thomas, G. Hunder, and D. Bloch, Overtraining in neural networks that interpret clinical data, *Clinical Chemistry*, 39, 1998-2004 (1993).
- 5 8. J. Furlong, M. Dupuy, and J. Heinsimer, Neural network analysis of serial cardiac enzyme data, *A.J.C.P.*, 96, 134-141 (1991).
9. W. Dassen, R. Mulleneers, J. Smeets, K. den Dulk, F. Cruz, P. Brugada, and H. Wellens, Self-
10 learning neural networks in electrocardiography, *J. Electrocardiol*, 23, 200-202 (1990).
10. E. Baum and D. Haussler, What size net gives valid generalization? *Advances in Neural Information Processing Systems*, Morgan Kauffman Publishers, San
15 Mateo, CA, 81-90 (1989).
11. A. Blum, *Neural Networks in C++*, John Wiley & Sons, New York, (1992).
12. S. Haykin, *Neural Networks A Comprehensive Foundation*, Macmillan College Publishing Company, New
20 York, (1994).
13. J. Swets, Measuring the accuracy of diagnostic systems, *Science*, 240, 1285-1293 (1988).
14. M. Zweig and G. Campbell, Receiver-operating characteristic (ROC) plots: a fundamental evaluation
25 tool in clinical medicine, *Clinical Chemistry*, 39, 561-577 (1993).
15. D. Bluestein, L. Archer, The sensitivity, specificity and predictive value of diagnostic information: a guide for clinicians, *Nurse
30 Practitioner*, 16, 39-45 (1991).
16. C. Schweiger, G. Soeregi, S. Spitzauer, G. Maenner, and A. Pohl, Evaluation of laboratory data by conventional statistics and by three types of
35 neural networks, *Clinical Chemistry*, 39, 1966-1971 (1993).

SUBSTITUTE SHEET (RULE 26)

-3-

Blood clots are the end product of a complex chain reaction where proteins form an enzyme cascade acting as a biologic amplification system. This system enables relatively few molecules of initiator products to induce sequential activation of a series of inactive proteins, known as factors, culminating in the production of the fibrin clot. Mathematical models of the kinetics of the cascade's pathways have been previously proposed.

10 In [1], a dynamic model of the extrinsic coagulation cascade was described where data were collected for 20 samples using quick percent, activated partial thromboplastin time (APTT), thrombin time (TT), fibrinogen, factor(F) II, FV, FVII, FX, 15 anti-thrombin III (ATIII), and factor degradation product (FDP) assays. These data were used as input to the model and the predictive output compared to actual recovered prothrombin time (PT) screening assay results. The model accurately predicted the PT result 20 in only 11 of 20 cases. These coagulation cascade models demonstrate: (1) the complexity of the clot formation process, and (2) the difficulty in associating PT clot times alone with specific conditions.

25 Thrombosis and hemostasis testing is the in vitro study of the ability of blood to form clots and to break clots in vivo. Coagulation (hemostasis) assays began as manual methods where clot formation was observed in a test tube either by tilting the tube or 30 removing fibrin strands by a wire loop. The goal was to determine if a patient's blood sample would clot after certain materials were added. It was later determined that the amount of time from initiation of the reaction to the point of clot formation in vitro 35 is related to congenital disorders, acquired disorders, and therapeutic monitoring. In order to

SUBSTITUTE SHEET (RULE 26)

-4-

remove the inherent variability associated with the subjective endpoint determinations of manual techniques, instrumentation has been developed to measure clot time, based on (1) electromechanical properties, (2) clot elasticity, (3) light scattering, (4) fibrin adhesion, and (5) impedance. For light scattering methods, data is gathered that represents the transmission of light through the specimen as a function of time (an optical time-dependent measurement profile).

Two assays, the PT and APTT, are widely used to screen for abnormalities in the coagulation system, although several other screening assays can be used, e.g. protein C, fibrinogen, protein S and/or thrombin time. If screening assays show an abnormal result, one or several additional tests are needed to isolate the exact source of the abnormality. The PT and APTT assays rely primarily upon measurement of time required for clot time, although some variations of the PT also use the amplitude of the change in optical signal in estimating fibrinogen concentration.

Blood coagulation is affected by administration of drugs, in addition to the vast array of internal factors and proteins that normally influence clot formation. For example, heparin is a widely-used therapeutic drug that is used to prevent thrombosis following surgery or under other conditions, or is used to combat existing thrombosis. The administration of heparin is typically monitored using the APTT assay, which gives a prolonged clot time in the presence of heparin. Clot times for PT assays are affected to a much smaller degree. Since a number of other plasma abnormalities may also cause prolonged APTT results, the ability to discriminate between these effectors from screening assay results may be clinically significant.

SUBSTITUTE SHEET (RULE 26)

-5-

Using a sigmoidal curve fit to a profile, Baumann, et al [4] showed that a ratio of two coefficients was unique for a select group of blood factor deficiencies when fibrinogen was artificially maintained by addition of exogenous fibrinogen to a fixed concentration, and that same ratio also correlates heparin to FII deficiency and FXa deficiencies. However, the requirement for artificially fixed fibrinogen makes this approach inappropriate for analysis of clinical specimens. The present invention makes it possible to predict a congenital or acquired imbalance or therapeutic condition for clinical samples from a time-dependent measurement profile without artificial manipulation of samples.

The present invention was conceived of and developed for predicting the presence of congenital or acquired imbalances or therapeutic conditions of an unknown sample based on one or more time-dependent measurement profiles, such as optical time-dependent measurement profiles, where a set of predictor variables are provided which define characteristics of profile, and where in turn a model is derived that represents the relationship between a congenital or acquired imbalance or therapeutic condition and the set of predictor variables (so as to, in turn, utilize this model to predict the existence of the congenital or acquired imbalance or therapeutic condition in the unknown sample).

SUMMARY OF THE INVENTION

The present invention is directed to a method and apparatus for predicting the presence of at least one congenital or acquired imbalance or therapeutic condition from at least one time-dependent measurement

SUBSTITUTE SHEET (RULE 26)

- 6 -

profile. The method and apparatus include a)
performing at least one assay on an unknown sample and
measuring a respective property over time so as to
derive a time-dependent measurement profile, b)
5 defining a set of predictor variables which
sufficiently define the data of the time-dependent
profile, c) deriving a model that represents the
relationship between a diagnostic output and the set
of predictor variables, and d) utilizing the model to
10 predict the existence of a congenital or acquired
imbalance or therapeutic condition in the unknown
sample relative to the diagnostic output. In one
embodiment, training data is provided by performing a
plurality of assays on known samples, the model is a
15 multilayer perceptron, the relationship between the
diagnostic output and the set of predictor variables
is determined by at least one algorithm, and the at
least one algorithm is a back propagation learning
algorithm. In a second embodiment of the present
20 invention, the relationship between the diagnostic
output and the set of predictor variables is derived
by a set of statistical equations.

Also in the present invention, a plurality of
time-dependent measurement profiles are derived, which
25 time-dependent measurement profiles can be optical
time-dependent measurement profiles such as ones
provided by a automated analyzer for thrombosis and
hemostasis, where a plurality of optical measurements
are taken over time, and where the plurality of
30 optical measurements are normalized. The optical
profiles can include one or more of a PT profile, a
fibrinogen profile, an APTT profile, a TT profile, a
protein C profile, a protein S profile and a plurality
of other assays associated with congenital or acquired
35 imbalances or therapeutic conditions.

SUBSTITUTE SHEET (RULE 26)

-7-

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a general neuron diagram relating to the embodiment of the present invention utilizing a neural network;

5 Figure 2 is a diagram of a multilayer perceptron for predicting congenital or acquired imbalances or therapeutic conditions, relating to the neural network embodiment of the present invention;

10 Figure 3 is an optical profile with first and second derivatives of a normal clotting sample;

Figure 4 is an illustration of two learning curves;

Figure 5 is an illustration of an unstable learning curve;

15 Figure 6 is a graph showing a comparison of training and cross-validation learning curves;

Figure 7 is a graph showing a comparison of training error for training tolerances of 0.0 and 0.1;

20 Figure 8 is a ROC illustrating the effect of decision boundary on classification;

Figure 9 is a Table comparing hidden layer size with prediction error;

25 Figure 10 is a receiver operator characteristic plot related to predicting an abnormality in relation to Factor VIII;

Figure 11 is a graph demonstrating the ability to predict actual Factor VIII activity;

30 Figure 12 is a receiver operator characteristic plot related to predicting an abnormality in relation to Factor X; and

SUBSTITUTE SHEET (RULE 26)

-8-

Figure 13 is a chart listing examples of predictor variables for use in the present invention.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

5 In the present invention, both a method and apparatus are provided for predicting the presence of at least one congenital or acquired imbalance or therapeutic condition. As one of the first steps of the method, one or more time-dependent measurements
10 are performed on an unknown sample. The term "time-dependent measurement" is referred to herein to include measurements derived from assays (e.g. PT, APTT, fibrinogen, protein C, protein S, TT, ATIII, plasminogen and factor assays). The terms "unknown
15 sample" and "clinical sample" refer to a sample, such as one from a medical patient, where a congenital or acquired imbalance or therapeutic condition associated with thrombosis/hemostasis is not known (or, if suspected, has not been confirmed). In the present
20 invention, a coagulation property is measured over time so as to derive a time-dependent measurement profile. In a preferred embodiment, the time-dependent measurement is an optical measurement for deriving an optical profile. For example, a PT
25 profile, a fibrinogen profile, a TT profile, an APTT profile and/or variations thereof can be provided where, an unknown sample is analyzed for clot formation based on light transmittance over time through the unknown sample. In another preferred
30 embodiment, two (or more) optical profiles are provided, such as both a PT profile and an APTT profile.

After the time-dependent measurement profiles are provided, a set of predictor variables are defined

SUBSTITUTE SHEET (RULE 26)

-9-

which sufficiently define the data of the time-dependent profile. One or more predictor variables comprise the set. And, in one embodiment, three or more, and in a preferred embodiment, four or more predictor variables were found to desirably make up the set. It was found that the characteristics of the time-dependent measurement profile could best be defined by one or more predictor variables, including the minimum of the first derivative of the optical profile, the time index of this minimum, the minimum of the second derivative of the optical profile, the time index of this minimum, the maximum of the second derivative, the time index of this maximum, the overall change in transmittance during the time-dependent measurement, clotting time, slope of the optical profile prior to clot formation, and slope of the optical profile after clot formation.

After defining the set of predictor variables, a model is derived which represents the relationship between a congenital or acquired imbalance or therapeutic condition and the set of predictor variables. This model can be derived from a neural network in one embodiment of the present invention. In another embodiment, the model is derived via a set of statistical equations.

Neural networks represent a branch of artificial intelligence that can be used to learn and model complex, unknown systems given some known data from which it can train. Among the features of neural networks that make them an attractive alternative for modeling complex systems are :

1. They can handle noisy data well and recognize patterns even when some of the input data are obscured or missing.

SUBSTITUTE SHEET (RULE 26)

-10-

2. It is unnecessary to determine what factors are relevant a priori since the network will determine during the training phase what data are relevant, assuming there are at least some meaningful parameters in the set.

Neural networks are formed from multiple layers of interconnected neurons like that shown in Figure 1. Each neuron has one output and receives input $i_1 \dots i_n$ from multiple other neurons over connecting links, or *synapses*. Each synapse is associated with a synaptic weight, w_j . An adder Σ or linear combiner sums the products of the input signals and synaptic weights $i_j * w_j$. The linear combiner output sum_i and θ_i (a threshold which lowers or a bias which raises the output) are the input to the activation function $f()$. The synaptic weights are learned by adjusting their values through a learning algorithm.

After deriving the model, whether based on neural networks or statistical equations, the model is utilized to predict the existence of a congenital or acquired imbalance or therapeutic condition in the unknown sample relative to the time-dependent measurement profile(s). As such, a congenital or acquired imbalance or therapeutic condition can be predicted. Conditions which can be predicted as being abnormal in the present invention can include, among others, a) factor deficiencies, e.g. fibrinogen, Factors II, V, VII, VIII, IX, X, XI and XII, as well as ATIII, plasminogen, protein C, protein S, etc., b) therapeutic conditions, e.g. heparin, coumadin, etc., and c) conditions such as lupus anticoagulant. In one embodiment of the present invention, the method is performed on an automated analyzer. The time-dependent measurement profile, such as an optical data profile, can be provided automatically by the

-11-

automated analyzer, where the unknown sample is automatically removed by an automated probe from a sample container to a test well, one or more reagents are automatically added to the test well so as to
5 initiate the reaction within the sample. A property over time is automatically optically monitored so as to derive the optical profile. The predicted congenital or therapeutic condition can be automatically stored in a memory of an automated
10 analyzer and/or displayed on the automated analyzer, such as on a computer monitor, or printed out on paper. As a further feature of the invention, if the predicted congenital or acquired imbalance or therapeutic condition is an abnormal condition, then
15 one or more assays for confirming the existence of the abnormal condition are performed on the automated analyzer. In fact, in a preferred embodiment, the one or more confirming assays are automatically ordered and performed on the analyzer once the predicted
20 condition is determined, with the results of the one or more confirming assays being stored in a memory of the automated analyzer and/or displayed on the analyzer.

25 EXAMPLE 1: Prediction of Heparin in Sample

This example shows a set of predictor variables that adequately describe screening assay optical profiles, develops an optimal neural network design, and determines the predictive capabilities of an
30 abnormal condition associated with thrombosis/hemostasis (in this case for the detection of heparin) with a substantial and well-quantified test data set.

-12-

Simplastin™ L, Platelin™ L, calcium chloride solution (0.025 M), imidazole buffer were obtained from Organon Teknika Corporation, Durham, NC, 27712, USA. All plasma specimens were collected in 3.2% or 3.8% sodium citrate in the ratio of one part anticoagulant to nine parts whole blood. The tubes were centrifuged at 2000 g for 30 minutes and then decanted into polypropylene tubes and stored at -80°C until evaluated. 757 specimens were prepared from 200 samples. These specimens were tested by the following specific assays: FII, FV, FVII, FVIII, FIX, FX, FXI, FXII, heparin, fibrinogen, plasminogen, protein C, and AT-III. Samples represented normal patients, a variety of deficiencies, and therapeutic conditions. Of the specimen population 216 were positive for heparin determined by a heparin concentration greater than 0.05 units/ml measured with a chromogenic assay specific for heparin. The remaining specimens, classified as heparin-negative, included normal specimens, a variety of single or multiple factor deficiencies, and patients receiving other therapeutic drugs. Positive heparin samples ranged to 0.54 units/ml.

PT and APTT screening assays were performed on each specimen utilizing two automated analyzers (MDA™ 180s) and multiple reagent and plasma vials (Organon Teknika Corporation, Durham NC 27712, USA) over a period of five days. When clot-based coagulation assays are performed by an automated optically-based analyzer such as the MDA 180, data are collected over time that represents the normalized level of light transmission through a sample as a clot forms (the optical profile). As the fibrin clot forms, the transmission of light is decreased. The optical profile was stored from each test.

SUBSTITUTE SHEET (RULE 26)

-13-

The network configuration chosen, a multilayer perceptron (MLP) maps input predictor variables from the PT and APTT screening assays to one output variable (see Figure 2) which represents a single
5 specified condition. A similar network was also employed for PT-only variables and APTT-only variables. This specific MLP consists of three layers: the input layer, one hidden layer, and the output layer.

10 A normal optical profile is shown in Figure 3. The set of predictor variables were chosen with the intent of describing optical profiles as completely as possible with a minimum number of variables. They are summarized in Table 1 where t is time from initiation
15 of reaction, T is normalized light transmission through the reaction mixture, and pv_{jk} is the k th predictor variable of assay j .

The predictor variables were scaled to values
20 between 0 and 1, based on the range of values observed for each variable for assay type k

$$i_j = f\left(pv_{jk}, (pv_{j,n,k})_{\min}, (pv_{j,n,k})_{\max}\right).$$

25 The input variable set includes $i_{1...7}$ for both a PT assay and APTT assay for each specimen. For known output variable values, heparin samples with results of greater than 0.05 units/ml were considered positive and assigned a value of 1 while
30 negative samples were assigned a value of 0.

As the ratio of training set sample to the number of weights in a network decreases, the

SUBSTITUTE SHEET (RULE 26)

-14-

probability of generalizing decreases, reducing the confidence that the network will lead to correct classification of future samples taken from the same distribution as the training set. Thus, small samples
5 sizes, then can lead to artificially high classification rates. This phenomenon is known as overtraining. In order to achieve a true accuracy rate of 80%, a guideline for the number of samples in the training set is approximately five times the
10 number of weights in the network. For most of this work, a 14-6-1 network was used, leading to an upward bound on the sample size of $O(450)$. To monitor and evaluate the performance of the network and its ability to generalize, a cross-validation set is
15 processed at the end of each training epoch. This cross-validation set is a randomly determined subset of the known test set that is excluded from the training set.

Once the input predictor variables and output
20 values were determined for all specimen optical profiles, the 757 sets of data were randomly distributed into two groups: 387 were used in the training set and 370 were used in the cross-validation set. These same two randomly determined sets were
25 used throughout all the experiments.

All synaptic weights and threshold values were initialized at the beginning of each training session to small random numbers.

The error-correction learning rule is an
30 iterative process used to update the synaptic weights by a method of gradient descent in which the network minimizes the error as pattern associations (known input-output pairs) in the training set are presented to the network. Each cycle through the training set
35 is known as an epoch. The order or presentation of the pattern associations was the same for all epochs.

SUBSTITUTE SHEET (RULE 26)

-15-

The learning algorithm consists of six steps which make up the forward pass and the backward pass. In the forward pass, the hidden layer neuron activations are first determined

5

$$h = F(iW1 + \theta_h)$$

where h is the vector of hidden-layer neurons, i the vector of input-layer neurons, $W1$ the weight matrix between the input and hidden layers, and $F()$ the activation function. A logistic function is used as the activation function

10

$$F(x) = \frac{1}{1 + e^{-x}}.$$

15

Then the output-layer neurons are computed

$$o = F(hW2 + \theta_o)$$

where o represents the output layer, h the hidden layer and $W2$ the matrix of synapses connecting the hidden layer and output layers. The backward pass begins with the computation of the output-layer error

25
$$e_o = (o - d),$$

where d is the desired output. If each element of e_o is less than some predefined training error tolerance vector TE_{tol} , than the weights are not updated during that pass and the process continues with the next pattern association. A training error tolerance of

30

SUBSTITUTE SHEET (RULE 26)

-16-

0.1 was used in all experiments unless otherwise specified. Otherwise, the local gradient at the output layer is then computed:

$$5 \quad g_o = \alpha(1 - o)e_o.$$

Next, the hidden-layer local gradient is computed:

$$g_h = h(1 - h)W2g_o.$$

10

Once the hidden layer error is calculated, the second layer of weights is adjusted

$$W2_m = W2_{m-1} + \Delta W2$$

15

where

$$\Delta W2 = \eta h g_o + \gamma \Delta W2_{m-1}.$$

20 is the learning rate, γ is the momentum factor, and m is the learning iteration. The first layer of weights is adjusted in a similar manner

$$W1_m = W1_{m-1} + \Delta W1$$

25

where

$$\Delta W1 = \eta i e + \gamma \Delta W1_{m-1}.$$

-17-

The forward pass and backward pass are repeated for all of the pattern associations in the training set, referred to as an epoch, 1000 times . At the end of each epoch, the trained network is applied to the cross-validation set.

Several methods were employed to measure the performance of the network's training. Error, E , for each input set was defined as

$$E = \sqrt{\frac{1}{N} \sum_{q=1}^N (d_q - o_q)^2}.$$

The learning curve is defined as the plot of E versus epoch. The percent classification, ϕ , describes the percent of the total test set (training and cross-validation) that is correctly classified based on some defined decision boundary, β . Receiver-Operating Characteristic (ROC) plots have also been utilized to describe trained networks' ability to discriminate between the alternative possible outcome states. In these plots, measures of sensitivity and specificity are shown for a complete range of decision boundaries. The sensitivity, or true-positive fraction is defined as

$$\text{sensitivity} = \frac{\text{true positive}}{\text{true positive} + \text{false negative}}$$

and the false-positive fraction , or (1-specificity) is defined as

SUBSTITUTE SHEET (RULE 26)

$$(1 - \textit{specificity}) = \frac{\textit{false positive}}{\textit{false positive} + \textit{true negative}}$$

These ROC plots represent a common tool for evaluating clinical laboratory test performance.

5

Using the test set described, experiments were performed to determine if the presence of heparin could be predicted with this method. First, experiments were conducted to determine optimal error-correction backpropagation learning parameters: (1) hidden layer size, (2) learning rate, and (3) momentum. Additional experiments were also conducted to compare the performance of networks based on PT and APTT assays alone with that of one combining the results of both, the effect of the training error tolerance, and the decision boundary selection.

Figure 9 shows the effect of the hidden layer size on the training and cross validation error and the percent correct classification for the optimal decision boundary, defined as the decision boundary which yielded the lowest total number of false positives and false negatives from the total test set. As the hidden layer size is increased, the error is decreased. However, the ability to generalize does not increase after a hidden layer size of 6. The most significant benefit in terms of both error and percentage correct classification is between 4 and 6. A hidden layer size of 6 was used for the remainder of the experiments.

A series of experiments were conducted with $\eta = \{0.01, 0.1, 0.5, 0.9\}$ and $\gamma = \{0.0, 0.1, 0.5, 0.9\}$. Figure 4 shows the learning curves for two of the best combinations of parameters. Figure 5 shows an example learning curve

SUBSTITUTE SHEET (RULE 26)

-19-

when the learning rate is so high it leads to oscillations and convergence to a higher E. In general, as $\eta \rightarrow 0$ the network converged to a lower E and as $\gamma \rightarrow 1$, the rate of convergence improved. As

5 $\eta \rightarrow 1$, the value of E converged too increased and oscillations increased. In addition, as $\eta \rightarrow 1, \gamma \rightarrow 1$ exacerbated the oscillations.

Figure 6 shows a comparison of the learning curve for the training set and cross-validation set for $\eta=0.5$ and $\gamma=0.1$. It is a primary concern when developing neural networks, and it has been previously shown that it is important to look not only at the error in the training set for each cycle, but also the

10 cross-validation error.

Figure 7 shows the learning curve $\eta=0.5$ and $\gamma=0.1$ and a learning tolerance of 0.0 and 0.1. These results suggest that a small learning tends to smoothen the convergence of the learning process.

Figure 8 shows the ROC plot for networks trained with the predictor variables from each of the two screening assays with that of them combined. In the single assay cases, the hidden layer size was 3. While using the data from one assay does lead to some

20 success, using the information from both assays makes a significant improvement in the ability of the network to correctly predict the presence of heparin. This graph indicates that a 90% true positive proportion can be achieved with a false positive

25 proportion of 15%. Using a single assay, a 60-70% true positive proportion can be achieved with a false positive proportion of approximately 15%.

30

-20-

EXAMPLE 2: Factor VIII

Similar tests were run as in Example 1. As can be seen in Figures 10 and 11, two training sessions were conducted for predicting a Factor VIII condition in an unknown sample. Figure 10 is a receiver operator characteristic plot related to predicting an abnormality in relation to Factor VIII. In Figure 10, everything below 30% activity was indicated as positive, and everything above 30% was indicated as negative. Cutoff values other than 30% could also be used. In this Example, the activity percentage has a known accuracy of approximately + or - 10%. In Figure 11, the actual percent activity was utilized as the output.

EXAMPLE 3: Factor X

As can be seen in Figure 12, the method of the present invention was run similar to that as in Example 2, where here an abnormality in Factor X concentration was predicted from unknown samples. Everything below 30% activity was indicated as positive, and everything above 30% was indicated as negative. Cutoff values other than 30% could also be used.

The results of the cross-validation sample sets throughout the experiments indicate that the sample size was sufficient for the network to generalize. While the random distribution of the training and cross-validation sets were held constant throughout the experiments presented, other distributions have been used. These distributions, while all yielding different results, still lead to the same general conclusion.

Many alternatives for or additions to the set of predictor variables were explored. This included coefficients of a curve fitted to the data profile,

SUBSTITUTE SHEET (RULE 26)

-21-

pattern recognition, and clot time-based parameters. Low order functions tend to lose information due to their poor fit, and high order functions tend to lose information in their multiple close solutions. Clot-based parameters, such as clot time, slope in the section prior to the initiation of clot formation, and afterwards, are often available, but not always (because in some samples, the clot time is not detectable). The successful results observed indicate that the set of predictor variables used are effective for predicting congenital or acquired imbalances or therapeutic conditions.

The optimization of the network learning algorithm's parameters made significant differences in its performance. In general, performance was best with low learning rates, high momentum rates, some small training error tolerance, and a hidden layer size approximately half of the size of the input layer.

It is to be understood that the invention described and illustrated herein is to be taken as a preferred example of the same, and that various changes in the method and apparatus of the invention may be resorted to, without departing from the spirit of the invention or scope of the claims.

-22-

WE CLAIM:

1. A method for predicting the presence of at least one congenital or acquired imbalance or therapeutic
5 condition associated with thrombosis/hemostasis from at least one time-dependent measurement profile, comprising:
 - a) performing at least one time-dependent measurement on an unknown sample and measuring a
10 respective property over time so as to derive a time-dependent measurement profile;
 - b) defining a set of a plurality of predictor variables which sufficiently define the data of the time-dependent measurement profile;
 - 15 c) deriving a model that represents the relationship between the congenital or acquired imbalance or therapeutic condition, and the set of predictor variables; and
 - d) utilizing the model of step c) to predict the
20 existence of the congenital or acquired imbalance or therapeutic condition in the unknown sample.
2. A method according to claim 1, wherein said at least one time-dependent measurement profile is at
25 least one optical profile.
3. A method according to claim 2, wherein said at least one optical profile is provided by an automated analyzer for thrombosis and hemostasis
30 testing.
4. A method according to claim 2, wherein a plurality of optical measurements at one or more wavelengths are taken over time so as to derive said
35 at least one optical profile, said optical

SUBSTITUTE SHEET (RULE 26)

-23-

measurements corresponding to changes in light scattering and/or light absorption in the unknown sample.

5 5. A method according to claim 2, wherein a plurality of optical measurements are taken over time so as to derive said at least one optical profile, and wherein said plurality of optical measurements are each normalized to a first optical measurement.

10

6. A method according to claim 3, wherein in step a) said at least one optical profile is provided automatically by said analyzer, whereby said unknown sample is automatically removed by an automated probe
15 from a sample container to a test well, one or more reagents are automatically added to said test well so as to initiate said property changes within said sample, and the development of said property over time is automatically optically monitored so as to derive
20 said optical data profile.

7. A method according to claim 6, wherein after step d), a predicted congenital or acquired imbalance or therapeutic condition is automatically stored in a
25 memory of said automated analyzer and/or displayed on said automated analyzer.

8. A method according to claim 6, wherein in step d), one or more assays for confirming the
30 existence of said congenital or acquired imbalance or therapeutic condition is automatically performed.

9. A method according to claim 8, wherein said one or more confirming assays are automatically
35 ordered and performed on said analyzer, with results

SUBSTITUTE SHEET (RULE 26)

-24-

of said one or more assays being stored in a memory of said automated analyzer and/or displayed on said analyzer.

5 10. A method according to claim 1, further comprising:

before step a), providing a set of data from known samples, which data is used in step c) for deriving said model.

10

11. A method according to claim 10, wherein said data from known samples is provided by performing a plurality of assays on said known samples.

15 12. A method according to claim 10, wherein said model of step c) is a neural network.

20 13. A method according to claim 1, wherein said relationship in step c) is determined via at least one automated algorithm.

25 14. A method according to claim 1, wherein in step a), a plurality of time-dependent measurement profiles are derived for use in step b).

30 15. A method according to claim 14, wherein said plurality of time dependent measurement profiles includes at least two profiles from assays initiated with PT reagents, APTT reagents, fibrinogen reagents and TT reagents.

16. A method according to claim 13, wherein said model is a multilayer perceptron, and wherein said at

SUBSTITUTE SHEET (RULE 26)

-25-

least one algorithm is a back propagation learning algorithm.

17. A method according to claim 1, wherein said
5 set of predictor variables includes a plurality of: a
minimum of the first derivative of the profile, a time
index of the minimum of the first derivative, a
minimum of the second derivative of the profile, a
time index of the minimum of the second derivative, a
10 maximum of the second derivative of the profile, a
time index of the maximum of the second derivative, an
overall change in the coagulation parameter during the
time-dependent measurement on the unknown sample, a
clotting time, a slope of the profile prior to clot
15 formation, and a slope of the profile after clot
formation.

18. A method according to claim 17, wherein
three or more of said predictor variables are within
20 said set.

19. A method according to claim 18, wherein more
than three of said predictor variables are within said
set.
25

20. A method according to claim 1, wherein said
unknown sample is a sample from a medical patient, and
wherein in step d), both said model and additional
patient medical data are utilized for predicting the
30 existence of said congenital or acquired imbalance or
therapeutic condition.

21. An apparatus for performing at least one
time-dependent measurement on an unknown sample to
35 derive at least one time-dependent measurement

-26-

profile, and predicting the presence of at least one congenital or acquired imbalance or therapeutic condition associated with thrombosis/hemostasis from the at least one time-dependent measurement profile, comprising:

means for performing at least one time-dependent measurement on an unknown sample and measuring a respective property over time so as to derive a time-dependent measurement profile;

means for defining a set of a plurality of predictor variables which sufficiently define the data of the time-dependent measurement profile;

means for deriving a model that represents the relationship between the congenital or acquired imbalance or therapeutic condition, and the set of predictor variables; and

means for utilizing the model of step c) to predict the existence of the congenital or acquired imbalance or therapeutic condition in the unknown sample.

22. An apparatus according to claim 21, wherein said means for performing at least one time-dependent measurement comprises an optical system for performing at least one optical measurement over time and so as to derive an at least one optical profile.

23. An apparatus according to claim 22, wherein said optical system is part of an automated analyzer for thrombosis and hemostasis testing.

24. An apparatus according to claim 22, wherein said optical means comprises a means for performing a plurality of optical measurements at one or more wavelengths over time so as to derive said at least

-27-

one optical profile, said optical measurements corresponding to changes in light scattering and/or light absorption in the unknown sample.

5 25. An apparatus according to claim 22, wherein
in said optical system, a plurality of optical
measurements are taken over time so as to derive said
at least one optical profile, and wherein said
plurality of optical measurements are each normalized
10 to a first optical measurement.

 26. An apparatus according to claim 23 which is
an automated analyzer for thrombosis and hemostasis
testing, and wherein said at least one optical profile
15 is provided automatically by said analyzer, whereby
said unknown sample is automatically removed by an
automated probe from a sample container to a test
well, one or more reagents are automatically added to
said test well so as to initiate said property changes
20 within said sample, and the development of said
property over time is automatically optically
monitored so as to derive said optical data profile.

 27. An apparatus according to claim 26, further
25 comprising at least one of a memory and a display
wherein a predicted congenital or acquired imbalance
or therapeutic condition is automatically stored in
said memory of said automated analyzer and/or
displayed on said display of said automated analyzer.

30

 28. An apparatus according to claim 26, further
comprising means for automatically performing one or
more assays for confirming the existence of said
congenital or acquired imbalance or therapeutic
35 condition.

SUBSTITUTE SHEET (RULE 26)

-28-

29. An apparatus according to claim 28, wherein said means for performing one or more confirming assays is an automatic performing means wherein said confirming assays are automatically ordered and performed on said analyzer, with results of said one or more assays being stored in a memory of said automated analyzer and/or displayed on a display of said analyzer.

10

30. An apparatus according to claim 21, further comprising means for providing a set of data from known samples, which data is used in step c) for deriving said model.

15

31. An apparatus according to claim 30, wherein said data from known samples is provided by said means for performing a plurality of assays on said known samples.

20

32. An apparatus according to claim 30, wherein said means for deriving a model is a means for deriving a model by means of a neural network.

25

33. An apparatus according to claim 21, wherein said relationship determined by said deriving means comprises a means for determining said relationship via at least one automated algorithm.

30

34. An apparatus according to claim 21, wherein said means for performing at least one time-dependent measurement is capable of performing a plurality of time-dependent measurement profiles.

SUBSTITUTE SHEET (RULE 26)

-29-

35. An apparatus according to claim 34, wherein said means for performing a plurality of time dependent measurement profiles includes a means for performing at least two profiles from assays initiated with PT reagents, APTT reagents, fibrinogen reagents and TT reagents.

36. An apparatus according to claim 33, wherein said model is a multilayer perceptron, and wherein said at least one algorithm is a back propagation learning algorithm.

37. An apparatus according to claim 21, wherein said set of predictor variables includes a plurality of: a minimum of the first derivative of the profile, a time index of the minimum of the first derivative, a minimum of the second derivative of the profile, a time index of the minimum of the second derivative, a maximum of the second derivative of the profile, a time index of the maximum of the second derivative, an overall change in the coagulation parameter during the time-dependent measurement on the unknown sample, a clotting time, a slope of the profile prior to clot formation, and a slope of the profile after clot formation.

38. An apparatus according to claim 37, wherein three or more of said predictor variables are within said set.

30

39. An apparatus according to claim 38, wherein more than three of said predictor variables are within said set.

-30-

40. An apparatus according to claim 21, wherein said unknown sample is a sample from a medical patient, and wherein said utilizing means comprising a means for utilizing both said model and additional
- 5 patient medical data for predicting the existence of said congenital or acquired imbalance or therapeutic condition.

SUBSTITUTE SHEET (RULE 26)

1 / 13

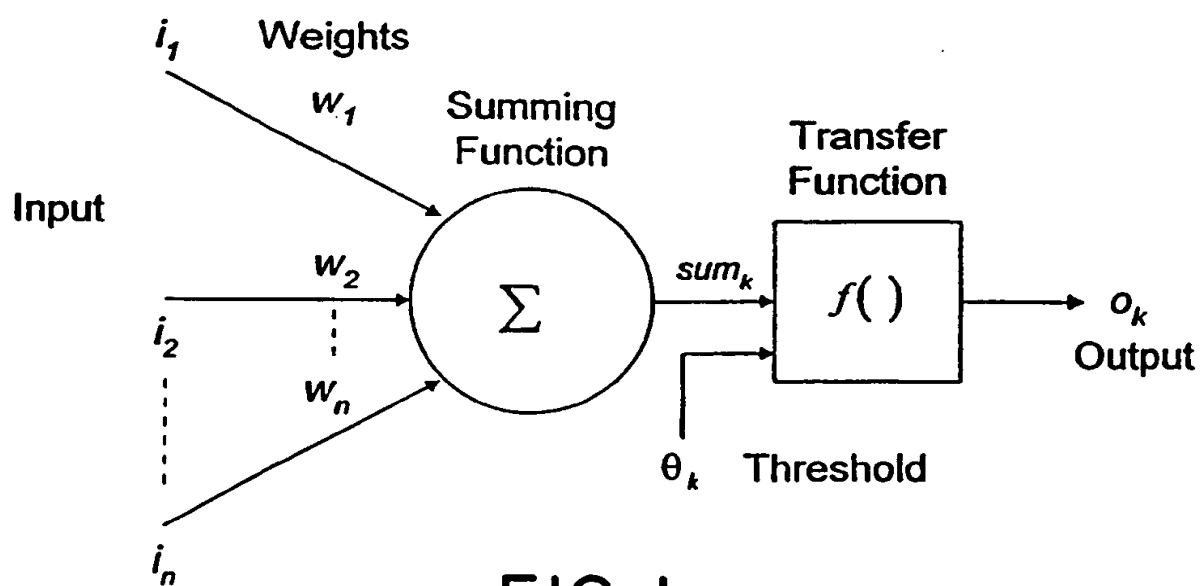
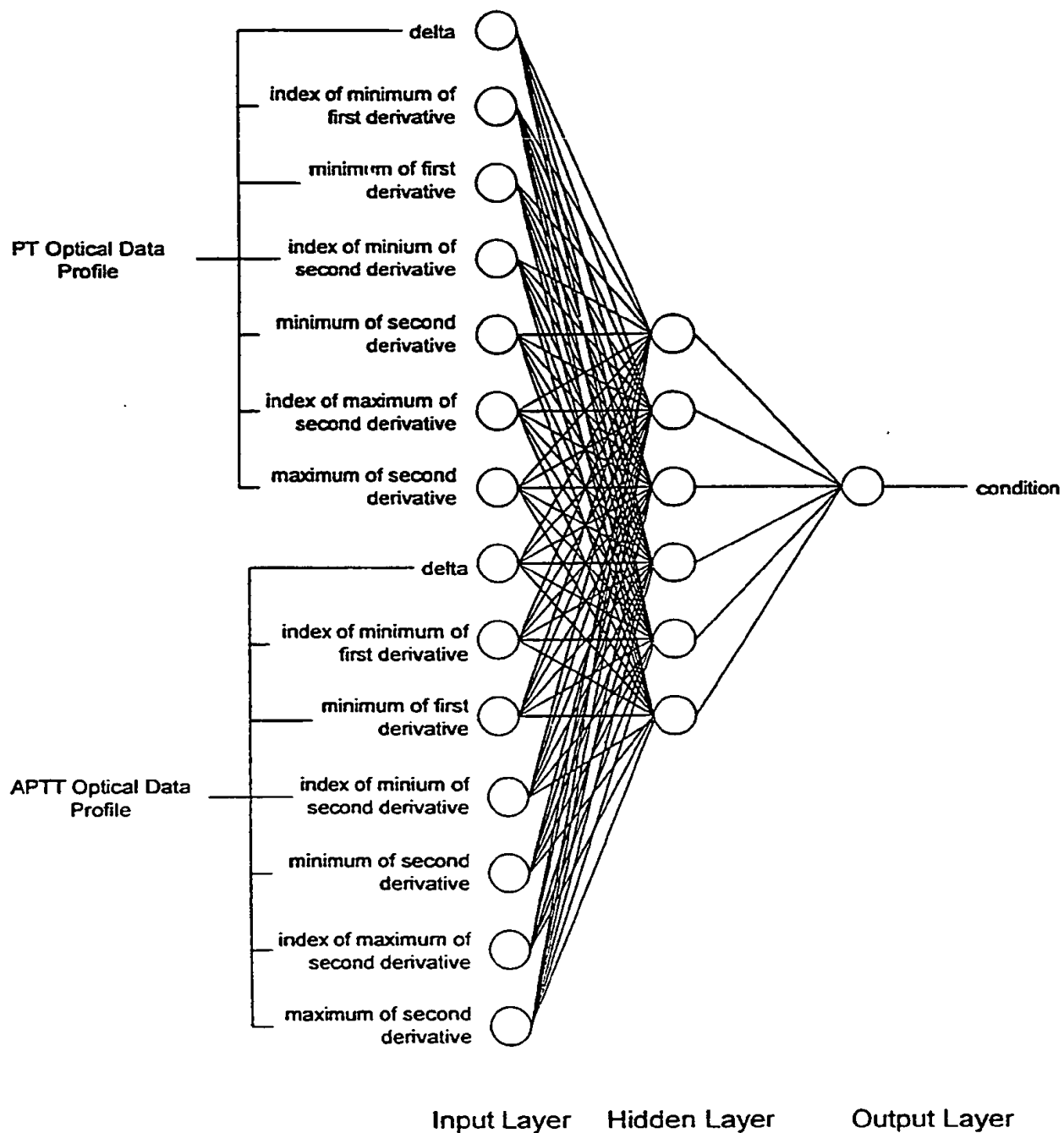


FIG. 1
Neuron Diagram

2 / 13

**FIG. 2**

Multilayer Perceptron for Predicting Congenital and Therapeutic Conditions

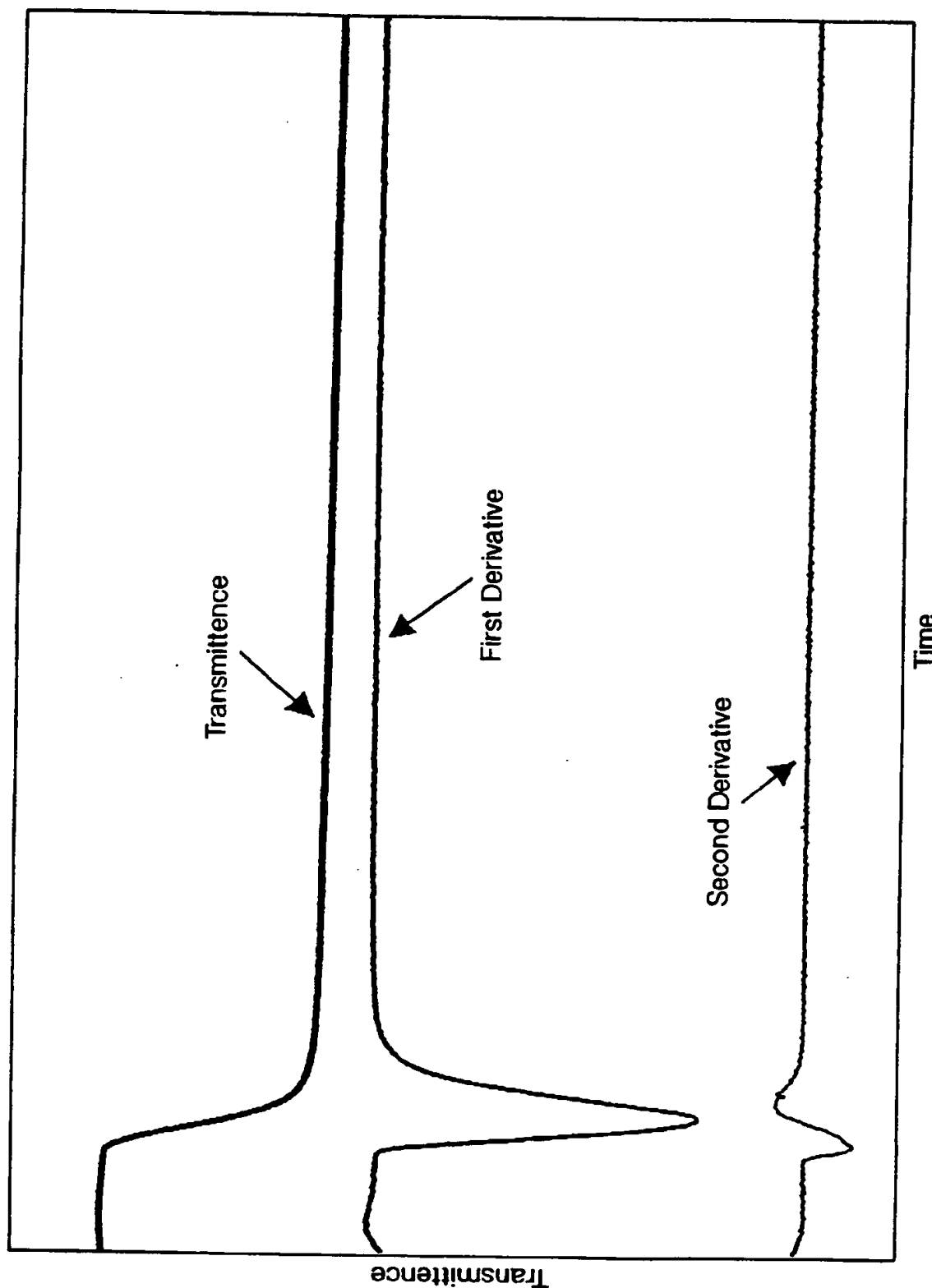


FIG. 3
Normal APTT Optical Profile with First and Second Derivative

4/13

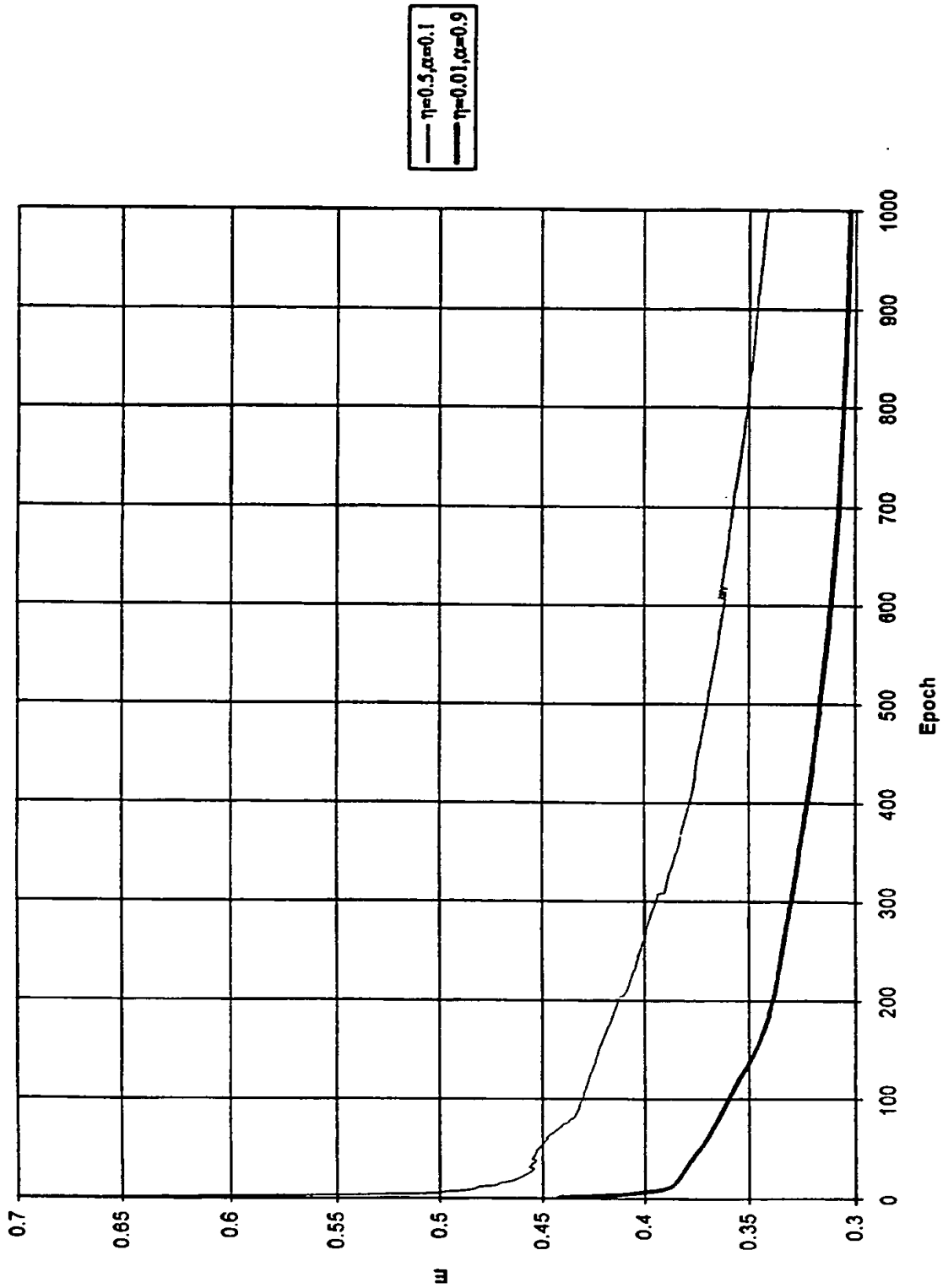


FIG. 4

Two of the Best Learning Curves

5/13

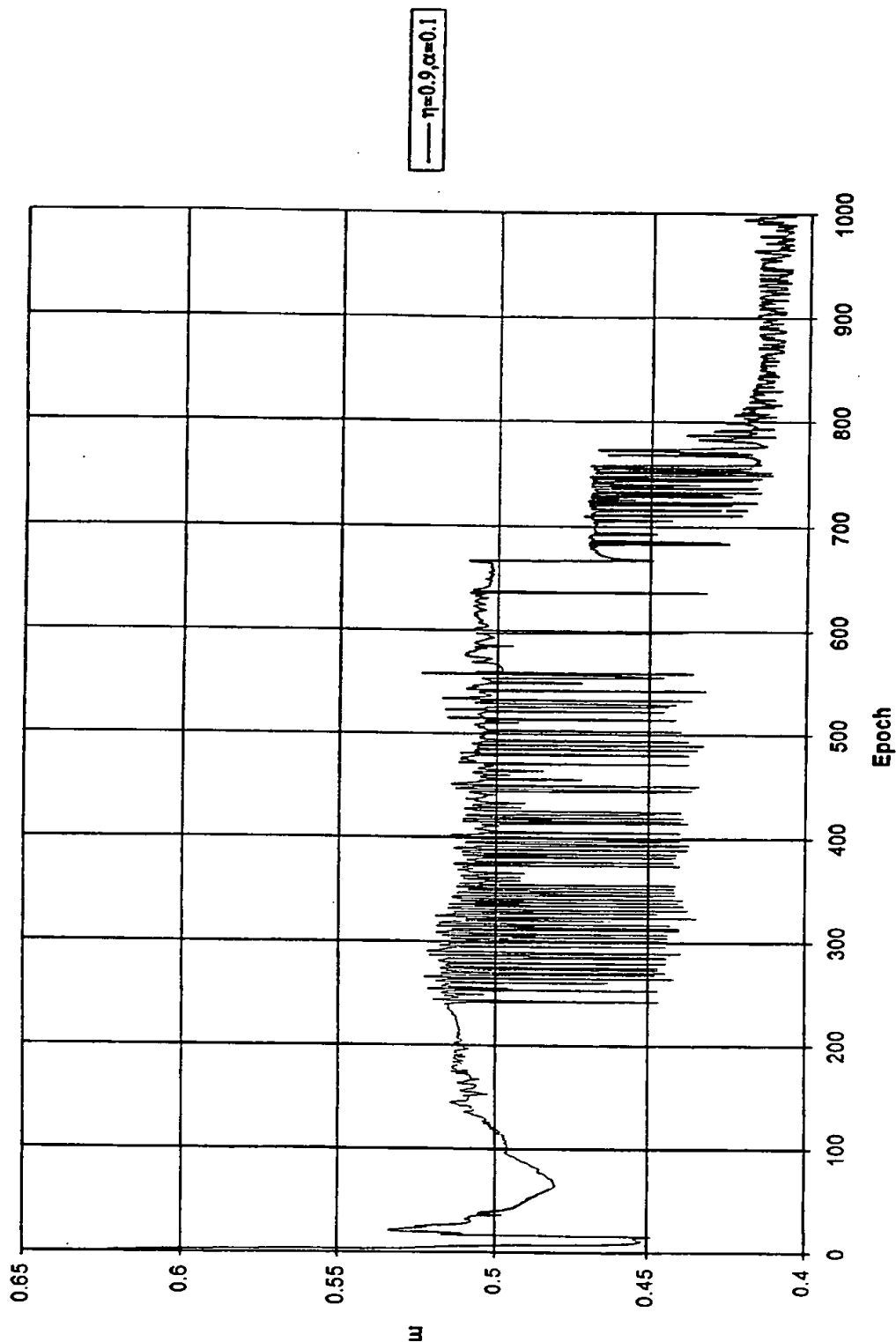


FIG. 5

One of the Most Unstable and Worst Learning Curves

SUBSTITUTE SHEET (RULE 26)

6 / 13

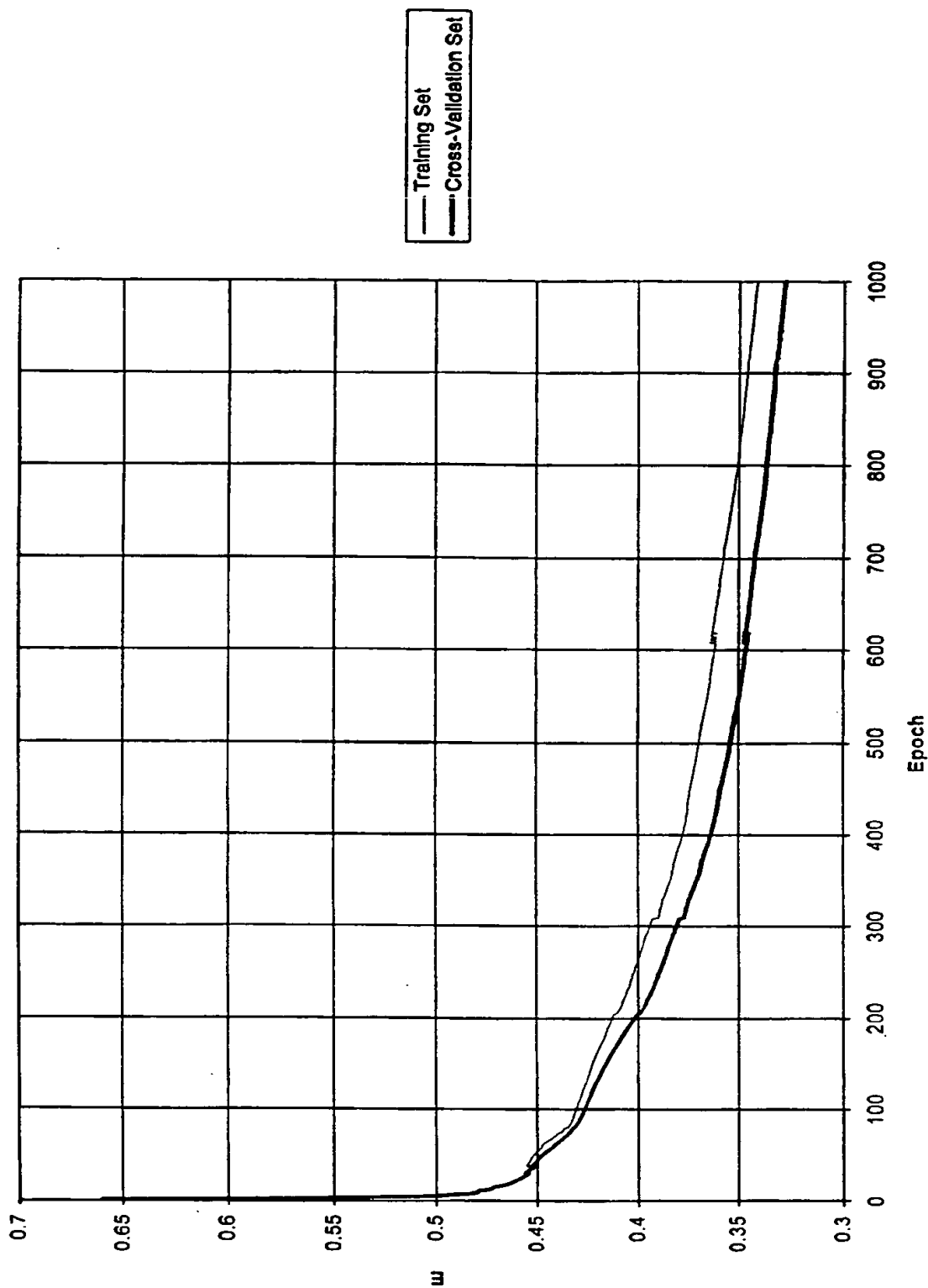
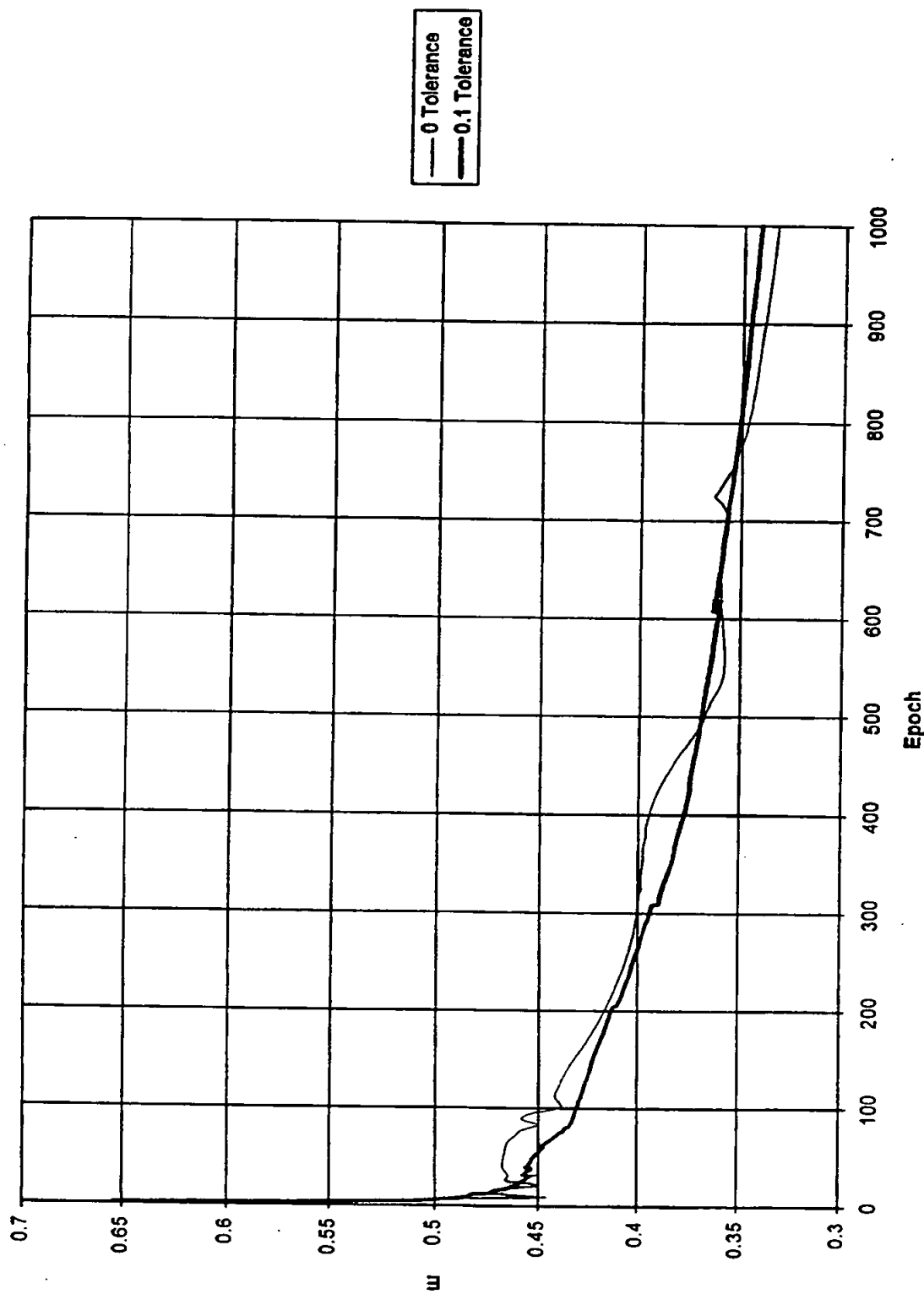


FIG. 6
Comparison of Training and Cross-Validation Learning Curves

SUBSTITUTE SHEET (RULE 26)

7/13

**FIG. 7**

Comparison of Training Error for Training Tolerances of 0.0 and 0.1

8 / 13

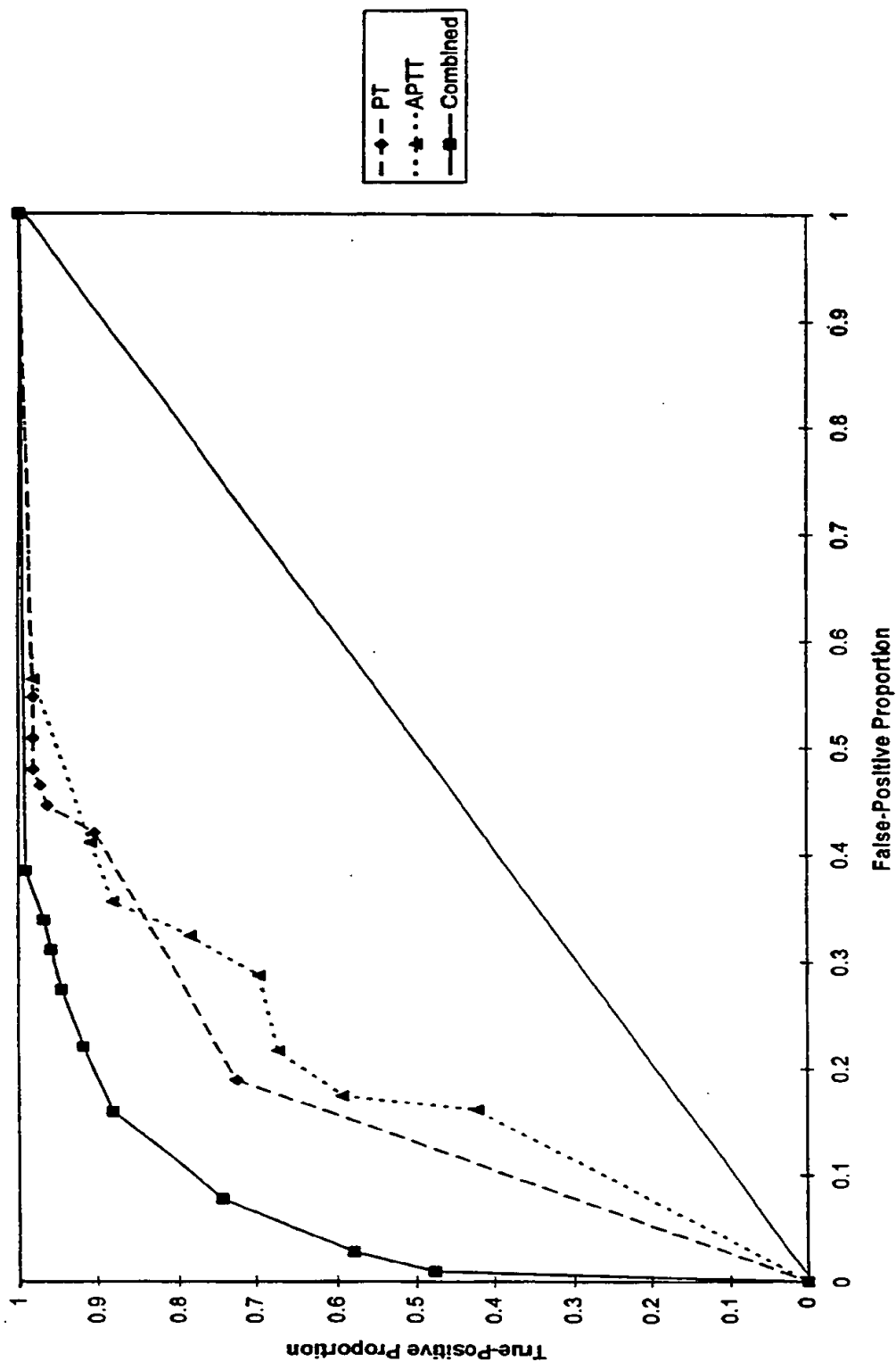


FIG. 8
Effect of Decision Boundary on Classification

Hidden Layer Size	Error		ψ_{ODB}
	E_{tr}	E_{cv}	
2	0.384	0.376	0.848
4	0.386	0.354	0.835
6	0.341	0.328	0.875
8	0.358	0.327	0.857
10	0.346	0.325	0.856
12	0.347	0.322	0.855

FIG. 9

Effects of Various Hidden Layer Sizes on Heparin Network Performance After 1000 Epochs

10 / 13

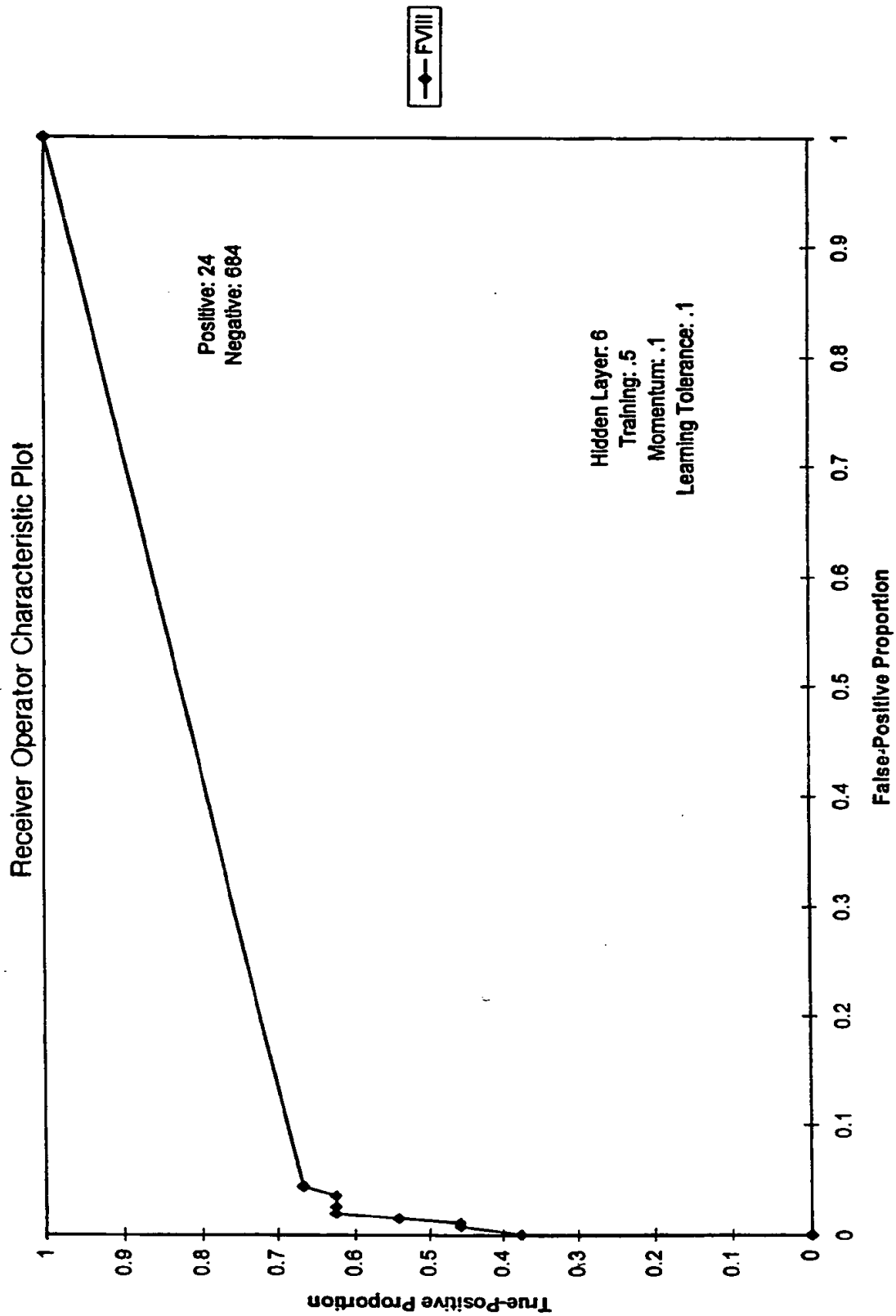


FIG. 10

11 / 13

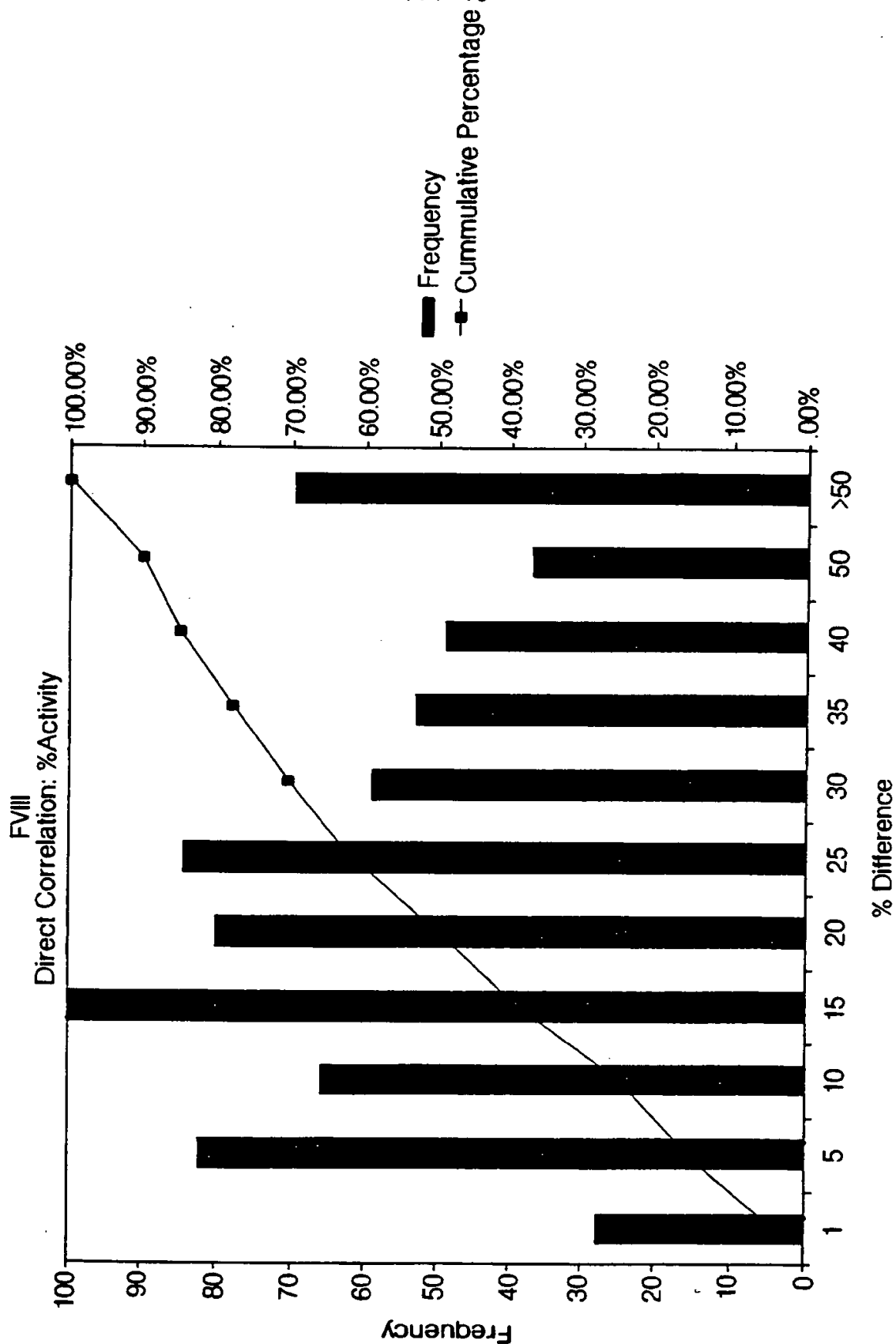


FIG. 11

12 / 13

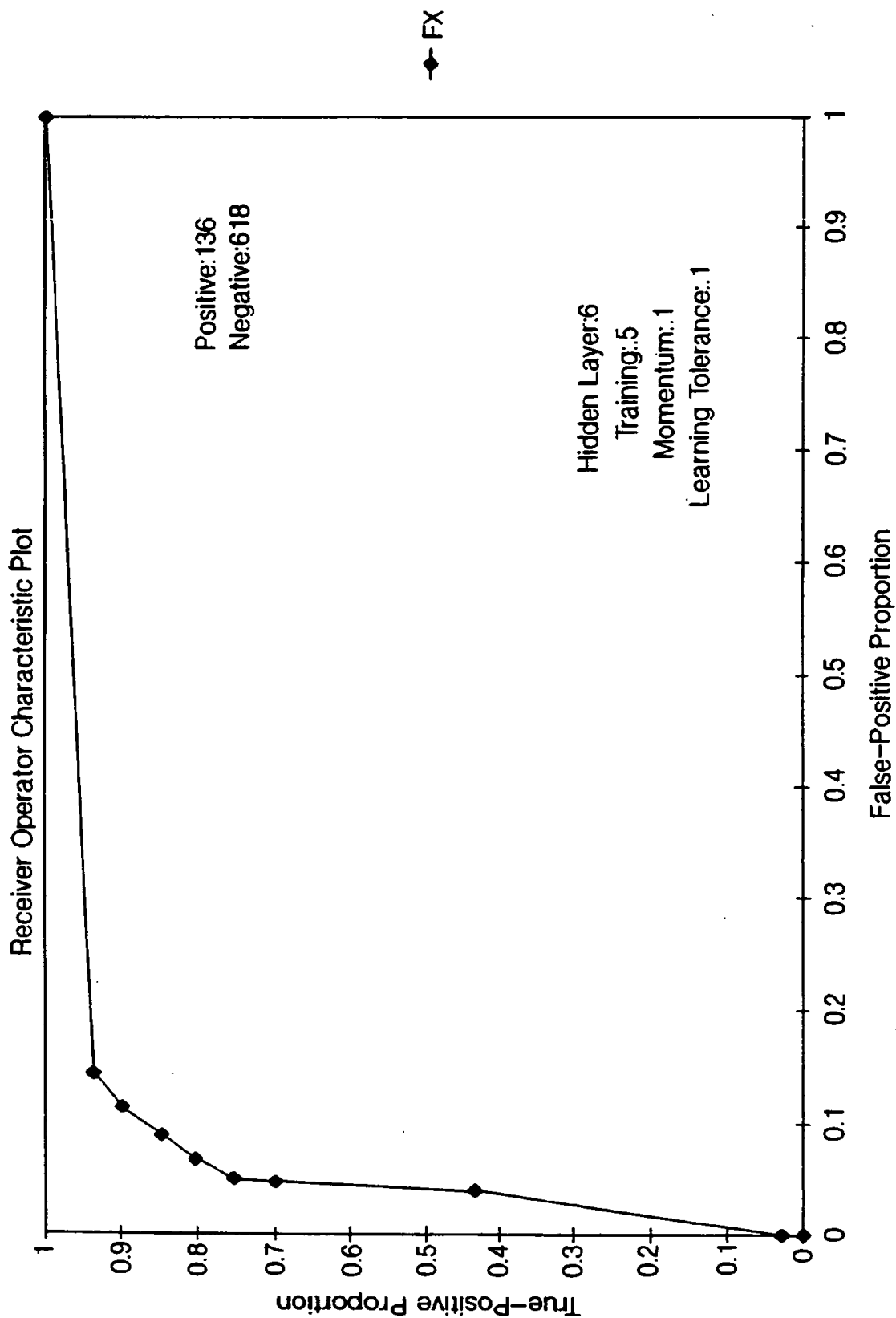


FIG. 12

Table 1. Predictor Variables

Predictor Variable	Description
$p_{v f 1} = \left(\frac{dT}{dt} \right)_{\min}$	minimum of the first derivative
$p_{v f 2} = t \text{ at } \left(\frac{dT}{dt} \right)_{\min}$	time index of the minimum of the first derivative
$p_{v f 3} = \left(\frac{d^2T}{dt^2} \right)_{\min}$	minimum of the second derivative
$p_{v f 4} = t \text{ at } \left(\frac{d^2T}{dt^2} \right)_{\min}$	index of the minimum of the second derivative
$p_{v f 5} = \left(\frac{d^2T}{dt^2} \right)_{\max}$	maximum of the second derivative
$p_{v f 6} = t \text{ at } \left(\frac{d^2T}{dt^2} \right)_{\max}$	index of the maximum of the second derivative
$p_{v f 7} = T_{f_0} - T_{f_n}$	overall change in transmittance during the reaction

FIG. 13

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/08905

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :G06F 19/00

US CL :364/496

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 364/496-499, 578, 413.01, 413.02, 413.07-413.09; 382/128, 133, 134; 436/50, 63, 69

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US, A, 5,156,974 (GROSSMAN ET AL) 20 OCTOBER 1992, see at least the Abstract.	1-40.
A	US, A, 4,998,535 (SELKER ET AL) 12 MARCH 1991, see at least the Abstract.	1-40.
A	US, A, 4,199,748 (BACUS) 22 APRIL 1980, see at least the Abstract.	1-40.

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be part of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

15 AUGUST 1996

Date of mailing of the international search report

09 OCT 1996

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

EMANUEL T. VOELTZ

Telephone No. (703)305-9714

Form PCT/ISA/210 (second sheet)(July 1992)*